

CERTIFICATE OF FILING

I hereby certify that this paper and every paper referred to therein as being enclosed is being filed with the USPTO via facsimile to the designated fax number (571) 273-8300 or via EFS-Web and addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date below

Date: January 2, 2009

By /Kurt G. Briscoe/
Kurt G. Briscoe

Attorney Docket No. 106985-2
Confirmation No. 4429

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT : Gerold SCHULER et al
SERIAL NO. : 10/618,134
CUSTOMER NO. : 27384
FILED : July 11, 2003
FOR : CD4⁺CD25⁻ T CELLS AND TR1-LIKE REGULATORY T CELLS
ART UNIT : 1644
EXAMINER : Amy Juedes

Hon. Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR §1.131

SIR:

We, the undersigned, hereby declare as follows:

1. We are the joint inventors of the subject matter disclosed and claimed in the above-identified application.

2. We completed the present invention in Germany prior to April 1, 2002.

3. To establish the date of completion of the invention claimed in this application, we submit true and correct reproductions of laboratory notebook entries for Experiment 336

(“E336”) as Exhibit A. Since these laboratory notebook entries are in the German language, we also submit a certified English language translation of these pages as Exhibit B.

4. From these laboratory notebook entries, it can be seen that the invention claimed in the above-identified application was made at least as early as March 2002.

5. The data from these laboratory notebook entries show that co-culture of CD4⁺CD25⁻ T cells with CD4⁺CD25⁺ T regulatory cells activated by stimulation with either anti-CD3/CD28 mAbs (graph on page 4) or mature allogenic DC (graph on page 6) anergized the CD4⁺CD25⁻ T cells, producing Tr-1-like regulatory cells that themselves exhibited suppressive properties.

6. The Tr-1 like regulatory cells exert their suppressive function via IL-10 as the addition of anti-IL-10 mAb abolished the suppressive function of these cells; see, again, the graphs on pages 4 and 6.

7. The suppressive function of CD4⁺CD25⁺ T regulatory cells, in contrast, was not abolished by the addition of anti-IL-10 mAb; see, again, the graphs on pages 4 and 6.

8. Fixation with formaldehyde also abolished the suppressive function of the Tr-1-like regulatory cells, whereas this was not the case for the CD4⁺CD25⁺ T regulatory cells; see, again, the graphs on pages 4 and 6.

9. The certified English language translation at various points indicates that certain words/entries could not be translated because they were illegible to the translator. These words/entries, although illegible to the translator, are legible to us from the original documents, and are translated as indicated in Exhibit C.

10. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and that the foregoing statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 18 Dec 2008

By G Schuler
Gerold Schuler

Dated: 18/Dec/2008

By Detlef Dieckmann
Detlef Dieckmann

EXHIBIT A

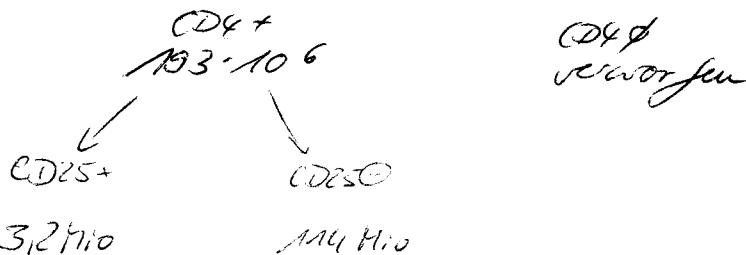
TO RULE 131 DECLARATION
OF THE INVENTORS

SCHULER ET AL.
USSN 10/618,134

20.03.02

* NAF von Lern 270080 einen gr. Teil
bekommen zu
freihit: 25 · 10⁸

* CD4+ Selektions - MACs



~ 0,8 Mio / well - ideal.

* 48 well Pl. 1x - vivo 20 C. 5%
2x well 1: CD4+ CD15 - 0,8 Mio } je 1 well mit
+ CD4+ CD15 + 0,8 Mio } Anti CD3/28
2x well 2: CD4+ CD15 + 0,8 Mio } 10% FBS
2x well 3: CD4+ CD26 - 0,8 Mio } je 1 well mit
R-DC E284 1:20

37 °C 0IN

* 96 well Pl. 1x - vivo 20 C. 5%
ca. 100 well 1 well + 1% 10410

22.03.02

* 48 well Pl. 1x - vivo 20 C. 5%
jetzt und freihit:

Anti CD3/28 R-DC

CD4+ CD25+ 1:1 11 Mio 1,1 Mio

CD4+ CD25+ 0,6 Mio 0,4 Mio

CD4+ CD25- 0,4 Mio 0,75 Mio

CD4+ CD25- R-DC: 10,6 Mio

* R-DC E284 R-DC jetzt: 1,6 Mio

* 96 well 1x - vivo 20 C. 5% Rg.

CD4+ CD15 - 1,6 Mio

CD4+ CD15 + 1:1 (CD3/28)

0,3 Mio

CD4+ CD15 + 1:1 (CD3/28) 0,3 Mio

- 9 - fix + 0,3 Mio

CD4+ CD15 - 1:1 (CD3/28) 0,3 Mio

CD4+ CD15 - 1:1 (CD3/28) 0,3 Mio

(22.03.02) * S6 UPI. 1x-1110 200. 5%. S. Reg. 116 mino
 CD4+CD15 ϕ refined 116 mino }
 CD4+CD15 ϕ 1+ 1:1 (R-DC) 0,3 mino } + RDX Exp.
 CD4+CD15 ϕ 1+ 1:1 (1-) fix 0,15 mino } 284
 CD4+CD15 ϕ (R-DC) 0,3 mino } 1:20
 CD4+CD15 ϕ (1- -1 fix 0,15 mino }
 + Anti T110 10 μ l/me in einje waas
 (Kleine MR - Photo well)

* fixieren je 0,3 mino - 0,5 mino + 0,2 min
 2x. Formaldehyd; 30 min 4°C
 waschen mit PBS

IVLR-Protokoll

Exp No.: 33 TC von: Can TC pro well: 50000 DC von:

3

	1	2	3	4	5	6	7	8	9	10	11	12
$CO_4 + CO_5$ (Ruhende)	A	—	—	$CO_4 + CO_5$	—	—	—	—	—	—	—	—
$CO_4 + CO_5$ (Reinig.)	B	$CO_4 + CO_5 + 1$ CO₂ + CO₃	$CO_4 + CO_5$									
$CO_4 + CO_5$ (Reinig.)	C	$CO_4 + CO_5 + CO_2 + CO_3$										
$CO_4 + CO_5$ (Reinig.)	D	$CO_4 + CO_5 + 1$ CO₂ + CO₃	$CO_4 + CO_5 + CO_2 + CO_3$									
$CO_4 + CO_5$ (Reinig.)	E	$CO_4 + CO_5 +$ CO₂ + CO₃										
$CO_4 + CO_5$ (Reinig.)	F	$CO_4 + CO_5 +$ CO₂ + CO₃										
$CO_4 + CO_5$ (Reinig.)	G	$CO_4 + CO_5 +$ CO₂ + CO₃										
$CO_4 + CO_5$ (Reinig.)	H	$CO_4 + CO_5 +$ CO₂ + CO₃										

Angesetzt:

H³-Zugabe: 26.03.02 Messung: 27.03.02 (3)

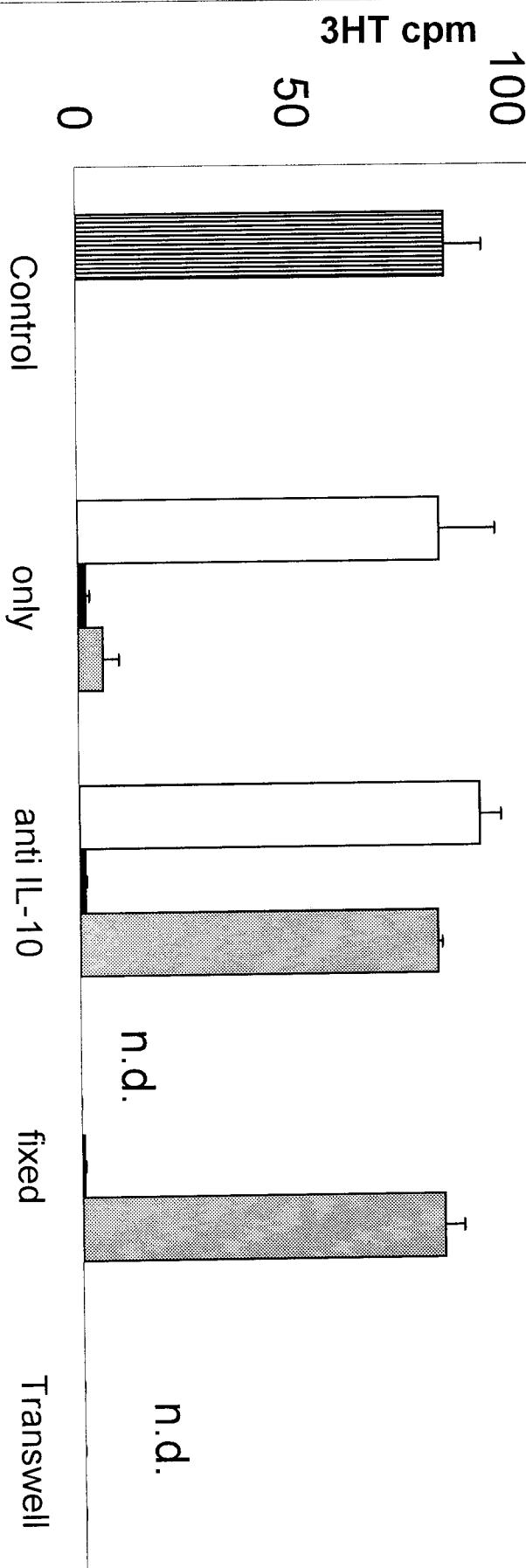
Exp. Beschreibung: + Ans. CO₃ 1041/mc 21.03.02

+ Ans. CO₁₀ — 22.03.02

Exp 336

Indicated Cells added to 10(5) CD4+CD25- T Cells
Stimulated with anti CD3/CD28

- CD4+CD25-
- CD4+CD25+
- ▨ CD4+CD25+/CD4+CD25- 1:1



II

MLR-Protokoll

Exp No.: 336 TC von: ~~Can~~ TC pro well: 50 000 DC von: ~~Erste~~

5

	1	2	3	4	5	6	7	8	9	10	11	12
CD4+ CD152 (Ruhende) R-DC 1:20	A	—	—	DC4+ corr + Th10 10 min								
CD4+ CD152 (Ruhende)	B	CD4+ corr + Th10 10 min	CD4+ corr									
R-DC 1:20	C	CD4+ corr + Th10 10 min	CD4+ corr	CD4+ corr + Th10 10 min								
CD4+ CD152 (Ruhende)	D	CD4+ corr + Th10 10 min	CD4+ corr	CD4+ corr + Th10 10 min								
R-DC 1:20	E	CD4+ corr + Th10 10 min	CD4+ corr	CD4+ corr + Th10 10 min								
CD4+ CD152 (Ruhende)	F	CD4+ corr + Th10 10 min	CD4+ corr + Th10 10 min	CD4+ corr + Th10 10 min								
R-DC 1:20	G	CD4+ corr + Th10 10 min	CD4+ corr + Th10 10 min	CD4+ corr + Th10 10 min								
CD4+ CD152 (Ruhende)	H	CD4+ corr + Th10 10 min	CD4+ corr + Th10 10 min	CD4+ corr + Th10 10 min								
R-DC 1:20												
R-DC 1:20												
R-DC 1:20												

Angesetzt: 22.03.02

H³-Zugabe: 26.03.02

Messung: 22.03.02

Exp. Beschreibung:

Exp 336

Indicated Cells added to 10(5) CD4+CD25- T Cells
Stimulated with mature allogeneic DC

- CD4+CD25-
- CD4+CD25+
- ▨ CD4+CD25+/CD4+CD25- 1:1

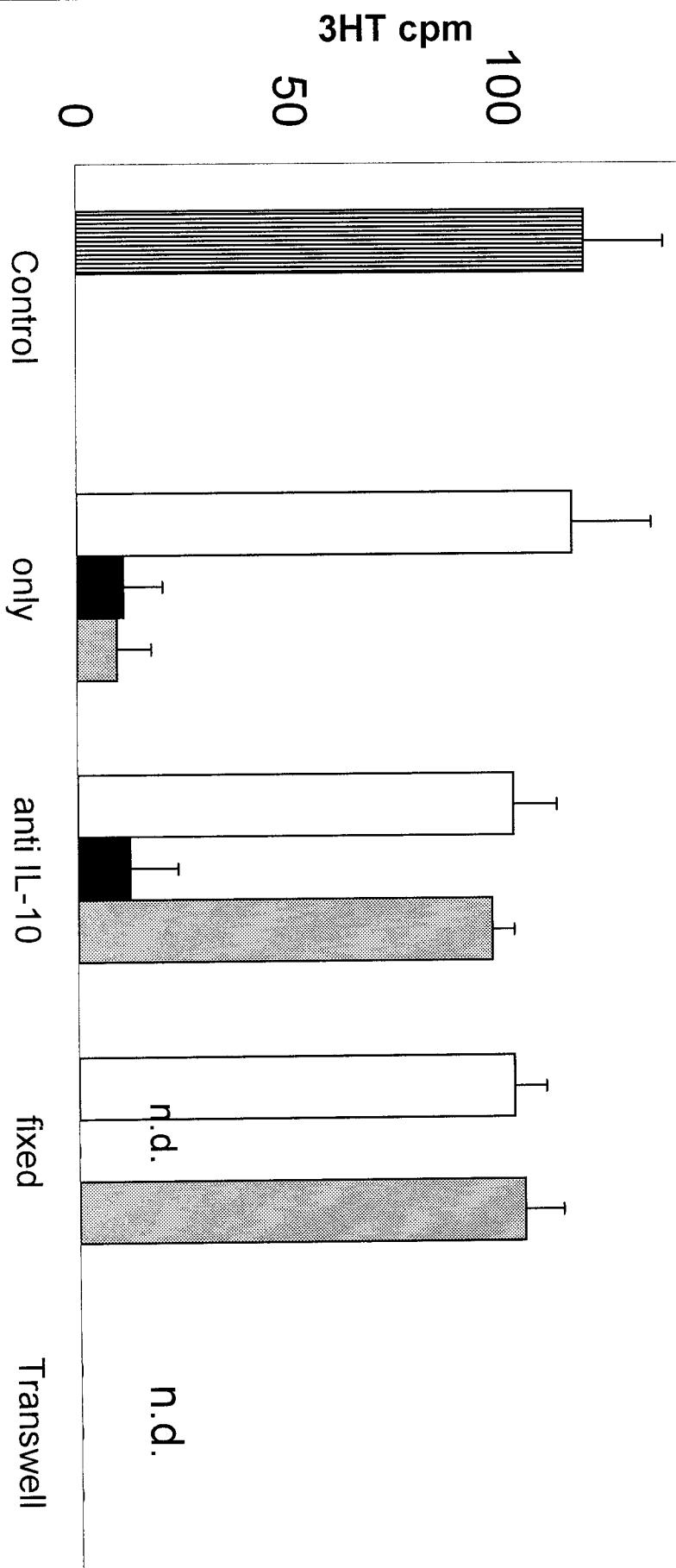


EXHIBIT B

TO RULE 131 DECLARATION
OF THE INVENTORS

SCHULER ET AL.
USSN 10/618,134



TRANSPERFECT

Certified Translation

ALBANY

AMSTERDAM

ATLANTA

AUSTIN

BARCELONA

BERLIN

BOSTON

BRUSSELS

CHARLOTTE

CHICAGO

DALLAS

DENVER

DUBAI

DUBLIN

FRANKFURT

GENEVA

HONG KONG

HOUSTON

IRVINE

LONDON

LOS ANGELES

MAMIA

MINNEAPOLIS

MONTREAL

MUNICH

NEW YORK

PARIS

PHILADELPHIA

PHOENIX

PORTLAND

RESEARCH

TRIANGLE PARK

SAN DIEGO

SAN FRANCISCO

SAN JOSE

SEATTLE

SINGAPORE

STOCKHOLM

SYDNEY

TOKYO

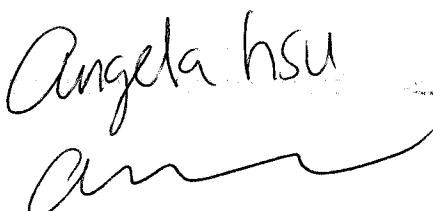
TORONTO

VANCOUVER

WASHINGTON, DC

I, Angela Hsu, hereby declare that the following document is to the best of my knowledge and belief, a true and accurate translation of the document "E336," standing for, on information and belief, "Experiment 336," from German into English.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punished by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: 

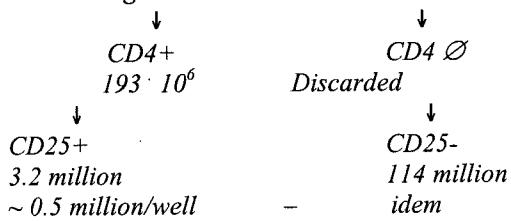
Date: November 14, 2008

03/20/02

*NAF received a big part of Leu 270080

Counted: 2.5×10^8

*CD4Ø negative selection MACS



* 48 well Pl. / x - vivo 20 c. 5% s.

2 x well 1 : CD4+ CD25Ø 0.8 million

+ CD4+ CD25+ 0.8 million

2 x well 2 : CD4+ CD25+ 0.8 million

2 x well 3 : CD4+ CD26- Ø 0.8 million

1 well each with

anti-CD3/28

10 µg/mL

1 well each with

R-DC E284

1:20

37°C 0/N

* [illegible] 6 well Pl. / x - vivo 20 c. 5% s

approx. 110 mL 1 well + [illegible] 10 u/mL

03/22/02

* 48 well Pl. of 03/20/02 tested and counted:

	Ab CD3/28	R-DC
CD4+ CD25+/- 1:1	1 million	1.1 million
CD4+ CD25+	0.6 million	0.4 million
CD4+ CD25 Ø	0.4 million	0.75 million

CD4+ CD25 Ø resting: 102.4 million

* [illegible] E284 R-DC defrosted: 1.6 million

* [illegible] v Pl. / x - vivo 20 c. 5% s. reg.

CD4+ CD25 Ø resting 1.6 million

CD4+ CD25+/- 1:1 (CD3/28) 0.3 million

CD4+ CD25+/- 1:1 (- "-) fix*¹ 0.15 million

CD4+ CD25+ (CD3/28) 0.3 million

- " - fix *¹ 0.15 million

CD4+ CD25 Ø (CD3/28) [illegible] 0.3 million

03/22/02	<p>* 96 v Pl. / <i>x</i> - vivo 20 c. 5% s. reg.</p> <table> <tbody> <tr> <td>CD4+ CD25 Ø resting</td><td>1.6 million</td></tr> <tr> <td>CD4+ CD25 Ø/+ 1:1 (R-DC)</td><td>0.3 million</td></tr> <tr> <td>CD4+ CD25 Ø/+ 1:1 (-“-) fix</td><td>0.15 million</td></tr> <tr> <td>CD4+ CD25+ (R/DC)</td><td>0.3 million</td></tr> <tr> <td>CD4+ CD25 Ø (R/DC)</td><td>0.3 million</td></tr> <tr> <td>CD4+ CD25 Ø - “ - fix</td><td>0.15 million</td></tr> </tbody> </table>	CD4+ CD25 Ø resting	1.6 million	CD4+ CD25 Ø/+ 1:1 (R-DC)	0.3 million	CD4+ CD25 Ø/+ 1:1 (-“-) fix	0.15 million	CD4+ CD25+ (R/DC)	0.3 million	CD4+ CD25 Ø (R/DC)	0.3 million	CD4+ CD25 Ø - “ - fix	0.15 million	<p>+ R-DC experiment 284 1:20</p>
CD4+ CD25 Ø resting	1.6 million													
CD4+ CD25 Ø/+ 1:1 (R-DC)	0.3 million													
CD4+ CD25 Ø/+ 1:1 (-“-) fix	0.15 million													
CD4+ CD25+ (R/DC)	0.3 million													
CD4+ CD25 Ø (R/DC)	0.3 million													
CD4+ CD25 Ø - “ - fix	0.15 million													
	<p>+ anti IL-10 10 µg/mL in some wells (no MLR [illegible] well)</p> <p>*¹ fixation 0.3 million – 0.5 million each + 0.2 mL 2X formaldehyde; 30 min. at 4°C wash with PBS</p>													

MLR protocol

Experiment number: 336 TC from: *Leu* TC per well: 50000 DC from: /

3

I

		1	2	3	4	5	6	7	8	9	10	11	12
<i>CD4+ CD25 Ø</i> (resting)	A			Ø									
<i>CD4+ CD25 Ø</i> (resting)	B				<i>CD4+ CD25+ / CD4+ CD25 Ø</i> <i>1:1</i>				<i>+ IL-10 10µg/mL</i>				
<i>CD4+ CD25 Ø</i> (resting)	C				<i>CD4+ CD25+ / CD4+ CD25 Ø</i> <i>1:1</i>				<i>CD4+ CD25 Ø</i> <i>too little fillegible</i>				
<i>CD4+ CD25 Ø</i> (resting)	D				<i>CD4+ CD25+ / CD4+ CD25 Ø</i> <i>1:1</i>								
<i>CD4+ CD25 Ø</i> (resting)	E				<i>CD4+ CD25+</i>								
<i>CD4+ CD25 Ø</i> (resting)	F				<i>CD4+ CD25+</i>								
<i>CD4+ CD25 Ø</i> (resting)	G				<i>+ IL-10 1µg/mL</i>								
<i>CD4+ CD25 Ø</i> (resting)	H				<i>CD4+ CD25 Ø</i>								

Prepared:

Addition of H3: 03/26/02 measurement: 03/27/02 3

Description of experiment:

+ *Anti CD3* 10 µg/mL 03/21/02
+ *Anti CD18* - " - 03/21/02

[see source for graph]

MLR protocol

III

Experiment number: 336 TC from: *Leu* [illegible] TC per well: 50000 DC from: E284

5

		1	2	3	4	5	6	7	8	9	10	11	12
CD4+ CD25 \emptyset (resting) R-DC 1:20	A			\emptyset					CD4+ CD25 \emptyset + IL-10 10 μ g/mL				
CD4+ CD25 \emptyset (resting) R-DC 1:20	B			CD4+ CD25+ / CD4+ CD25 \emptyset 1:1					CD4+ CD25 \emptyset fixed				
CD4+ CD25 \emptyset (resting) R-DC 1:20	C			CD4+ CD25+ / CD4+ CD25 \emptyset 1:1 + IL-10 μ g/mL									
CD4+ CD25 \emptyset (resting) R-DC 1:20	D			CD4+ CD25+ / CD4+ CD25 \emptyset 1:1 fixed									
CD4+ CD25 \emptyset (resting) R-DC 1:20	E			CD4+ CD25+ fixed									
CD4+ CD25 \emptyset (resting) R-DC 1:20	F			CD4+ CD25+ + IL-10 10 μ g/mL									
CD4+ CD25 \emptyset (resting) R-DC 1:20	G			CD4+ CD25+ fixed too few cells									
CD4+ CD25 \emptyset (resting) R-DC 1:20	H			CD4+ CD25 \emptyset									

Prepared: 03/22/02

Addition of H3: 03/26/02

measurement: 03/27/02 4

Description of experiment:

[see source for graph]

EXHIBIT C

**TO RULE 131 DECLARATION
OF THE INVENTORS**

**SCHULER ET AL.
USSN 10/618,134**

Date	Illegible German Word/Entry	Translation
3/20/02 before "6 well"	9	9
3/20/02 before "10 µ/mL"	IC-2	IC-2
3/22/02 before "E284"	1 284 vials	1 vial
3/22/02 before "v Pl."	96	96
3/22/02 after "(CD3/28)"	fix	fixed